THE JANE COFFIN CHILDS MEMORIAL FUND FOR MEDICAL RESEARCH

333 Cedar Street

New Haven, Conn.

APPLICATION FOR GRANT-IN-AID OF RESEARCH

1. Applicant: L. S. BARON, Ph.D. 28 October 1960

2. Chief, Department of Bacterial Immunology

3. Division of Immunology
Walter Reed Army Institute of Research
Washington 12, D. C.

4. "Studies on the Genetics of Sex and Virulence in Salmonella"

5. Total: $4400.

Permanent Equipment:

Binocular Phase Contrast Microscope and Photographic Attachment	$1800.
Photomicrographic Exposure Meter	120.
Cailloux Micromanipulator	770.
Electrophotometer	350.
Micro-Waring Blendor Cups	260.
Ultraviolet Lamps	100.
Binocular Dissecting Microscope	300.

Travel:

Consultation and attendance at scientific meetings	700.

6. Period of Investigation: 1961 - 1962.

7. A. Specific Aims:

1) To obtain strains of Salmonella typhosa and Salmonella typhimurium having easily identifiable genetic markers and which are capable of acting as genetic donors (F^+, Hfr male bacteria).

2) To study the inheritance of Salmonella genes by strains of other enteric bacteria, especially Escherichia coli.

3) To investigate possible genetic determinants of virulence in the Salmonella group.

B. Plan of Procedure and Outline of Methods:

Previous studies in this laboratory have established a number of genetic homologies between Escherichia and Salmonella by means of conjugation between Hfr E. coli and F^- Salmonella. The interactions between the Salmonella chromosome and the injected Escherichia genome have been analyzed by investigations of stable and unstable diploid heterozygote recombinants. We hope to extend these previous studies to the analysis of recombinants obtained by conjugation between F^+ and Hfr Salmonella and F^- Escherichia and Shigella. These recombinants will be analyzed for the inheritance of biochemical, nutritional, antigenic and phage determinants.

Our primary goal is to obtain information on the genetic determinants of virulence in Salmonella species. We have already reported that diploid heterozygotes of Salmonella typhosa obtained by conjugation with Hfr Escherichia are significantly less virulent for mice. Progress in understanding the nature of the genetic determinants of virulence should be greatly accelerated if it is possible to transfer virulence from Salmonella to Escherichia. An especially useful system should be the use of Salmonella typhimurium Hfr and F^- Escherichia. S. typhimurium is a natural pathogen of mice and one organism injected intraperitoneally is capable of causing death. On the other hand, as many as 10^8 Escherichia injected intraperitoneally can not cause death of the host. Titration of challenge suspensions of various classes of Salmonella-Escherichia hybrids in mice would be the procedure used to localize virulence characters to short segments of the chromosome.

A closer analysis of chromosomal virulence determinants could then be afforded by means of phage-mediated transduction.

The basic methods for obtaining donor (F^+, Hfr) strains of bacteria have already been established by a number of workers. Using these technics we have isolated several donor strains of Salmonella typhosa. While these strains have proved useful we feel that it is desirable to build a stock of a number of Salmonella donor strains so that a variety of potentially useful genetic markers will be available.

The donor strains of Salmonella that we now have at hand show several differences from the F^+ and Hfr strains of Escherichia coli usually employed. These differences include orientation of transformed genetic elements, transmissability, and genetic stability. We therefore intend to study F^+ and Hfr

strains of Salmonella with respect to these characteristics by means of microscopic electrophoresis, immunological technics and studies on conjugation kinetics.

8. Salaries of the principal investigator, three co-workers and one technical assistant are provided for by the Walter Reed Army Institute of Research (approximate total: $35,000.)

Satisfactory supplies of experimental animals (mice, guinea pigs, and rabbits) and consumable supplies are available.

The Department of Bacterial Immunology possesses a reasonable amount of laboratory space supplied with constant temperature incubators, centrifuges, and other routine bacteriologic research equipment.

9. None.

10. Baron, Louis S. Jan. 2, 1924

B.S.	City College of New York	1947	Chemistry
M.S.	University of Illinois	1948	Bacteriology
Ph.D.	University of Illinois	1951	Bacteriology

Teaching Assistant, Dept. Bact., Univ. of Ill. 1949-1952
Dept. Bact. Immunol., WRAIR, 1952-1956
Chief, Dept. Bacterial Immunology, Division of Immunology, 1956-

Fellowships: E. R. Squibb & Sons Fellowship, 1951
President's Fellowship, Soc. Am. Bact., 1957

Societies: Sigma Xi, AAAS, Soc. Am. Bact., Amer. Assoc. Immun., Amer. Acad. Microbiol., Genetics Soc. Amer., Soc. Gen. Microbiol., Soc. Exptl. Biol. & Med., Biophysical Soc.

Positions: Member, Genetics Study Section, USPHS, National Institutes of Health, Bethesda, Md.
Member, Subcommittee on Maintenance of Genetic Stocks (Bacteria, Viruses) N.S.F.
Special Lecturer in Microbial Genetics, Dept. of Microbiology, George Washington Univ. School of Medicine, Wash. D.C.

11. Prof. J. Lederberg, Dept. of Genetics, Stanford Univ. Medical School, Palo Alto, Calif.
Prof. E. Englesberg, Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, Pa.
Prof. V. Bryson, Institute of Microbiology, Rutgers Univ., New Brunswick, N.J.

12. Baron, L. S., Formal, S. B., and Spilman, W., "Vi Phage-Host Interaction in Salmonella Typhosa". J. Bacteriol. 69:177-183, 1955.

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Kline, E. S., and Baron, L. S., "Xylose Metabolism in Genetic Variants of Salmonella Typhosa". Arch. Biochem. Biophys. 66:128-139, 1957.

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Baron, L. S., Carey, W. F., Spilman, W. M., "Genetic Recombination between Escherichia Coli and Salmonella Typhimurium". Proc. Natl. Acad. Sci. 45:976-984, 1959.

Englesberg, E., and Baron, L. S., "Mutation to Rhamnose Resistance and Transduction to Rhamnose Utilization in Salmonella Typhosa". J. Bacteriol. 78: 675-686, 1959.

Baron, L. S., Spilman, W. M., Carey, W. F., "Hybridization of Salmonella Species by Mating with Escherichia Coli". Science, 130:566-567, 1959.

Mandel, A. D., Baron, L. S., and Buckler, C. E., "Role of the Vi Antigen in Salmonella Paratyphi C Infection in Mice". Proc. Soc. Exptl. Biol. Med. 100: 653-656, 1959.

Muschel, L. H., Carey, W. F., and Baron, L. S., "Formation of Bacterial Protoplasts by Serum Components". J. Immunol. 82:38-42, 1960.

Baron, L. S., Carey, W. F., and Spilman, W. M., "Characteristics of a High Frequency of Recombination Strain of Salmonella Typhosa Compatible with Escherichia, Salmonella, and Shigella Species". Proc. Natl. Acad. Sci. 45:1752-1757, 1959.

Carey, W. F., Muschel, L. H., and Baron, L. S., "Formation of Bacterial Protoplasts In Vivo". J. Immunol. 84:183-188, 1960.

Baron, L. S., Spilman, W. M., and Carey, W. F., "Diploid Heterozygous Hybrids from Matings between Escherichia Coli and Salmonella Typhosa". J. Exptl. Med. 112:361-372, 1960.

Brinton, C. C., and Baron, L. S., "Transfer of Piliation from Escherichia Coli to Salmonella Typhosa by Genetic Recombination". Biochim. Biophys. Acta 42: 298-311, 1960.

Schneider, H., Formal, S. B., and Baron, L. S., "Experimental Genetic Recombination In Vivo between Escherichia Coli and Salmonella Typhimurium". Bacteriol. Proc. 1960.

Formal, S. B., Schneider, H., Bohner, H., and Baron, L. S., "Virulence of a Nutritional Mutant of Vibrio Comma". Proc. Soc. Exptl. Biol. Med. 103:359-361, 1960.

Buckler, C. E., Hansen, P. A., and Baron, L. S., "Linked Transduction of L-Fucose and D-Arabinose Utilization in Salmonella". Bacteriol. Proc. 1960.

Baron, L. S., and Formal, S. B., "Immunization Studies with Living Vaccine of Salmonella Typhimurium". Proc. Soc. Exptl. Biol. Med. 104:565-567, 1960.

Carey, W. F., Spilman, W. M., and Baron, L. S., "Genetic Transfer between Salmonella Typhosa and Serratia Marcescens". Bacteriol. Proc. 1960.

13. Additional remarks:

This is a request for some items of permanent equipment and for travel, both of which are important for the continued progress of these studies at the maximal rate. At the present moment, funds for these purposes are not available because of budgetary limitations.

The phase contrast microscope, exposure meter, and micromanipulator are essential items of equipment for the study of the segregation patterns of diploid heterozygous hybrids. The electrophotometer, Waring blendor cups and ultraviolet lamps are required for proposed studies on growth rates, kinetics of conjugation and production of mutant stocks.

While a number of research groups are engaged in the study of genetic recombination in bacteria, their efforts have not extended to the study of the genetic basis of virulence. Our adequate laboratory animal rooms as well as our previous experience in this phase of experimental work presents us with an almost unique opportunity for pursuit of this approach; availability of the needed equipment will assure results sooner, and the travel funds will permit consultation with colleagues and the presentation of these results to the scientific community.

14. Application approved by Dean or other administrative officer of the institution.

Signature _____

Title _____

15. Signature of applicant _____